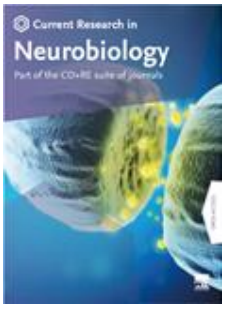
Peer Review Overview



Manuscript Title: Regulation of neuronal excitability by reactive oxygen species and calcium signaling: Insights into brain aging

Received	Feb 17, 2021
1st Decision	Mar 19, 2021
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Accepted	Apr 09, 2021

1st Decision letter

Reference: CRNEUR-D-21-00008
Title: Regulation of neuronal excitability by reactive oxygen species and calcium signaling: Insights into brain aging
Journal: Current Research in Neurobiology

Dear Mr. Hoerndli,

Thank you for submitting your Graphical Review manuscript to Current Research in Neurobiology.

I have completed my evaluation of your manuscript. The two expert reviewers recommend reconsideration of your manuscript following minor revision and modification. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Apr 18, 2021.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

Current Research in Neurobiology values your contribution and I look forward to receiving your revised manuscript.

Kind regards,

Anna S Mitchell, Ph.D.
Editor in Chief
Current Research in Neurobiology

Comments from Editors and Reviewers:

Reviewer #1:

This review article by Doser and Hoerndli is timely and informative. The illustrations are visually appealing. I have only a few suggestions for the author's consideration at their discretion.
1) Third highlight and abstract: consider mentioning the direction of modulation of calcium by ROS (or of calcium homeostasis), i.e. up or down. Actually, I would recommend trying to include the direction whenever words like "modulates", "changes", "alter", or "regulates" are used here throughout. This will be of much help to the reader. In cases where the regulation can be bi-directional, stating this will help too.
2) Intro: Mentioning that aging is a major risk factor for neurological disorders is appropriate, but doing so in the first sentence makes it appear like this is a focus of the review. Thus, it might be better to instead start with the "normal" direct effects of aging on cognition and plasticity, i.e. even without any specific neurological disorder (as this seems to be the focus of the review, and appropriately so). Or make the focus on "normal aging" clear in a different manner.
3) The authors mention that LTP is reduced with aging. To my understanding, the is true for LTP induced by theta-burst stimulation (TBS) but not for LTP induced by high-frequency stimulation (HFS; 1-4x 1 sec at 100 Hz). However, while the HFS-LTP in young and adult rodent is dependent on NMDARs and not VDCCs, this appears to be the other way around in aged rodents. This appears to fit somewhat with what the authors describe in the current manuscript regarding iGluRs versus VDCCs with aging. Thus, the authors might want to include some discussion of this (depending on how they view the corresponding literature).

Reviewer #2:

This review presents a succinct summary of the possible interplay of calcium and ROS in the modulation of glutaminergic signaling. There are a few minor issues the authors should address:

1) When referencing the C. elegans studeies (e.g., Doser et al, 2020), the authors should clarify that worms do not have voltage-gated sodium channels, which might lead to calcium having a larger effect om neuromodulation in this organism than in others.
2) The manuscript does not have page numbers, so I will just suggest that the statement "These identified roles for this interplay provide means by which neuronal activity can regulate energy production while also preventing excitotoxicity from an overabundance of cytoplasmic calcium." be explained in more detail - it is unclear what they mean by regulation of energy production.
3) I am unsure of the validity of this statement: "Age-dependent ROS elevations that occur independent of calcium are also possible, but we are not aware of evidence for such an occurrence." There is an abundance of studies showing increased ROS in aging contexts, and most do not relate this to calcium changes. Granted, many studies have not looked for calcium changes, but this does not mean that they happened.

1st Author Response Letter

Response to comments from Editors and Reviewers:

First, we would like to thank both reviewers and the editor for the thorough review of this manuscript. In addition to addressing the reviewers' suggestions (detailed in the sections below), we made some minor changes that improve the flow of the manuscript and reader understanding. These changes include renumbering of the informational panels in Figure 2 to match the order in which they are mentioned in the text, and to terminology at lines 57, 89, 256-257 and 286 that were thought to be more appropriate or clear.

Comments from Reviewer 1

1. Third highlight and abstract: consider mentioning the direction of modulation of calcium by ROS (or of calcium homeostasis), i.e. up or down. Actually, I would recommend trying to include the direction whenever words like "modulates", "changes", "alter", or "regulates" are used here throughout. This will be of much help to the reader. In cases where the regulation can be bi-directional, stating this will help too.

   We agree that distinguishing the directionality of the change in calcium due to oxidation would make the relation between ROS and calcium levels less ambiguous. However, the directionality is either increased or decreased depending on the calcium source ROS is acting on. For this succinct review, we were unable to mention exactly how ROS impacts the function of the many channels contributing to cytoplasmic calcium influx and efflux. We added an additional sentence at lines 205-208 to make it clear that ROS impacts calcium sources differentially. We also added the word "bidirectional" when describing the modulation of calcium by ROS (see highlight #2 and lines 192, 254).

2. Intro: Mentioning that aging is a major risk factor for neurological disorders is appropriate, but doing so in the first sentence makes it appear like this is a focus of the review. Thus, it might be better to instead start with the "normal" direct effects of aging on cognition and plasticity, i.e. even without any specific neurological disorder (as this seems to be the focus of the review, and appropriately so). Or make the focus on "normal aging" clear in a different manner.

   We agree with the reviewer that the introductory sentence was misleading. We replaced the first sentence at line 43 with one that is more focused on the impact of aging on cognitive abilities in general.

3. The authors mention that LTP is reduced with aging. To my understanding, the is true for LTP induced by theta-burst stimulation (TBS) but not for LTP induced by high-frequency stimulation (HFS; 1-4x 1 sec at 100 Hz). However, while the HFS-LTP in young and adult rodent is dependent on NMDARs and not VDCCs, this appears to be the other way around in aged rodents. This appears to fit somewhat with what the authors describe in the current manuscript regarding iGluRs versus VDCCs with aging. Thus, the authors might want to include some discussion of this (depending on

how they view the corresponding literature).

The findings on age-related changes in LTP are very mixed and unfortunately, it is not as clear-cut as LTP being hindered at aged synapses when elicited with TBS but not HFS because there are reports (albeit fewer of them) showing HFS-LTP is impaired with aging (Watson et al., 2002; Lucas et al., 2013). We do agree that these variations are worth mentioning, so we included a brief discussion at lines 134-142 that touch on the differences in age-related LTP data in relation to their stimulation protocols. We also agree that a discussion on the switch to a VGCC-dependent LTP mechanism in aging is relevant, so we included a sentence at lines 144-148 that outlines the known change in neuronal physiology that results an increased threshold for LTP in aging.

Comments from Reviewer 2

1. When referencing the C. elegans studies (e.g., Doser et al, 2020), the authors should clarify that worms do not have voltage-gated sodium channels, which might lead to calcium having a larger effect om neuromodulation in this organism than in others.

   Some of the aspects of the calcium-dependent signaling regulating glutamate receptor transport that we have outlined using C. elegans has been confirmed in vertebrate neurons (Hangen et al., 2018), so we didn't feel it was necessary to mention this when discussing parts of that regulation that have been observed in vertebrate studies. However, the regulation of glutamate receptor transport by a ROS-calcium interplay has only been seen by our lab in C. elegans, so we made note of this following the discussion of this mechanism at lines 212-215.

2. The manuscript does not have page numbers, so I will just suggest that the statement "These identified roles for this interplay provide means by which neuronal activity can regulate energy production while also preventing excitotoxicity from an overabundance of cytoplasmic calcium." be explained in more detail - it is unclear what they mean by regulation of energy production.

   We clarified what about energy production is being regulated by neuronal activity by adding "the rate of" in the phrase at lines 216- 217.

3. I am unsure of the validity of this statement: "Age-dependent ROS elevations that occur independent of calcium are also possible, but we are not aware of evidence for such an occurrence." There is an abundance of studies showing increased ROS in aging contexts, and most do not relate this to calcium changes. Granted, many studies have not looked for calcium changes, but this does not mean that they happened.

   We agree with the reviewer that the sentence is not valid. We point out at lines 230-233 ways in which ROS is increased in aging that may be independent of calcium levels. Because of this, we thought it was best to just remove the sentence.

References:

Lucas, D., Delgado-García, J.M., Escudero, B., Albo, C., Aza, A., Acín-Pérez, R., Torres, Y., Moreno, P., Enríquez, J.A., Samper, E., Blanco, L., Fairén, A., Bernad, A., Gruart, A., 2013. Increased Learning and Brain Long-Term Potentiation in Aged Mice Lacking DNA Polymerase µ. PLoS One 8, 1–17. https://doi.org/10.1371/journal.pone.0053243

Watson, J.B., Khorasani, H., Persson, A., Huang, K.-P., Huang, F.L., O'Dell, T.J., 2002. Age-related deficits in long-term potentiation are insensitive to hydrogen peroxide: Coincidence with enhanced autophosphorylation of Ca2+/calmodulin-dependent protein kinase II. J. Neurosci. Res. 70, 298–308. https://doi.org/https://doi.org/10.1002/jnr.10427

Accept Letter

Dear Mr. Hoerndli,

Thank you for submitting your manuscript to Current Research in Neurobiology.

I am pleased to inform you that your manuscript has been accepted for publication. Congratulations.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Current Research in Neurobiology and hope you will consider us again for future submissions.

Kind regards,

Anna S Mitchell, Ph.D.
Editor in Chief
Current Research in Neurobiology

Editor and Reviewer comments:

Reviewer #1: The authors have sufficiently addressed my comments (which were only suggestions for the author's consideration in the first place).

Reviewer #2: The authors have addressed my concerns about this review.

-------- End of Review Comments --------